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Control of citrinin production through plant latex

(citrinin/plant latex/fungistatic)

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ABSTRACT Effect of latex of different plants on growth and citrinin production by *Penicillium citrinum* was studied. Latex of *Ficus benghalensis*, *Tabernaemontana divaricata* and *Vallaris solanacea* comp etely suppressed the citrinin production by *P. citrinum*, while latex of *Thevetia puruviana* was not effective. The plant latex exhibited differential toxicity towards the growth and citrinin production.

Citrinin, a lemon yellow toxin, produced by *Penicillium citrinum* is reported to cause enlarged turbid kidney in rats with a degeneration and dilation of the lower nephrons and renal lesions resembling glomerulonephrosis[1,2]. It has been reported as a natural contaminant of rice, wheat, corn, rye, barley and oats[3-5]. Though use of fungicides and pesticides[6], volatile compounds[7,8], fatty acids[9], plant extracts[10,11], antibiotics[12] and food preservatives[13] have been suggested to check the growth of mycotoxin producing fungi and mycotoxin production, no attempt has been made to try plant latex in the control of citrinin production by *P. citrinum*. Hence, the present investigations were undertaken.

Latex of different plants (Table 1) was collected in a sterilized test tube by giving small incision. 2 ml of latex was added aseptically to 25 ml of medium (KNO_3 10 g ; KH_2PO_4 5 g ; $MgSO_47H_2O$ 2.5 g ; Sucrose 35 g ; $FeCl_2$ traces; distilled water 1000 ml) and subjected to steam sterilization for three consecutive days, 30 min. each day. These flasks were inoculated by 7 days old cultures of *P. citrinum* and incubated at 27±2°C for 15 days. At the end of the incubation period, the cultures were harvested on weighed Whatman filter paper No. 42. The filter papers alongwith mycelium was dried at 60–70°C for two days and weighed to a constant weight after cooling in a desiccator to determine the growth rate of the fungus. The citrinin was extracted and estimated from the culture filtrate as described earlier[14].

Latex of different plants was toxic to growth and citrinin production by *P. citrinum*. However, the degree of toxicity varied with the plant (Table 1). Latex of *Vallaris solanacea*, *Tabernaemontana divaricata* and *Ficus benghalensis* have completely suppressed the citrinin production. The latex of *Holarrhena antidysentrica* and *Carica papaya* also inhibited the citrinin synthesis to a significant degree. On the other hand, *Euphorbia tirucalli* and *Thevetia puruviana* inhibited the citrinin synthesis only partially. The inhibitory effect of latex of rest of the plants varied.

Ficus benghalensis, *Calotropsis gigantea*, *Carica papaya* and *Holarrhena antidysentrica* inhibited the vegetative growth of *P. citrinum* significantly, while

TABLE 1

Effect of plant latex on vegetative growth and citrinin production by *P. citrinum*.

Name of the Plant	Dry weight (in mg)	Citrinin (in ppb)	Final pH
Calotropis gigantea Linn. R. Br.	025.5	053.0	4.0
Carica papaya Linn.	029.3	017.6	5.8
Cryptostegia grandiflora R. Br.	095.4	085.4	4.4
Euphorbia tirucalli Linn.	126.8	106.1	4.9
Ficus benghalensis Linn.	014.3	–	4.8
Ficus racemosa Linn	123 4	035.3	3.0
Holarrhena antidysenirica (Roth) A DC	035.3	014.2	5.1
Plumeria rubra Linn.	115.2	030.8	4 2
Tabernaemontana divercata Linn.	042.5	–	4.0
Thevetia puruviana jus. ex steud.	132.8	123 8	4.2
Vallaris solanacea (Roth) O. Ktze	045.0	–	4.0
Control	151.3	230.0	6.0

Thevetia puruviana and *Euphorbia tirucalli* had marginal toxicity. Present observations are in agreement with those of Khare and Dhingra[15] and Saxena and Saksena[16] who also reported the fungistatic nature of papaya latex.

Thanks are due to Prof. L. L. Narayana for providing facilities.

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Influence of some heavy metals on pollen germination in *Argemone mexicana* L.

(*Argemone mexicana* L./heavy metals/pollen germination)

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ABSTRACT The influence of some heavy metal salts in the medium at low concentrations on germination and pollen tube growth of *Argemone mexicana* L., a road side weed species has been studied. Both pollen germination and pollen tube growth were retarded by the heavy metal salts. Nickel is most toxic, copper is least toxic and bismuth and lead are intermediary in their toxicity.

Presence of excessive amounts of heavy metals in the growing medium causes damage to plant cells, the degree and extent of injury being dependent on the concentration of the metal present. Toxicity imposed by heavy metals involves an overall disruption in the synchronization of different metabolic processes occurring in the cells, the resultant effect being manifest in the inhibition of the cell division and consequent retardation of growth[1]. Changes in the activities of some enzymes have been noticed to be brought in by the action of heavy metals. Toxic metals are capable of causing a reduction in the activity of hydrolases, *viz.*, α-amylase, phosphatase, RNase and protease in germinating seeds while the activities of catalase, peroxidase, IAA oxidase and ascorbic acid oxidase undergo considerable stimulation[1]. The activity of RNase was stimulated by nickel while that of α-amylase, phytase and ATPase was reduced in rice seedlings[2].

Studies have indicated that lead is accumulated in plants by absorption from soil, deposition through automobile exhausts, mining and smelting operations and due to various other industries in which lead is used as a catalyst or is otherwise involved in production[3]. The present study is intended to study the effect of various heavy metals like $Cu(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Bi(NO_3)_2$. *Argemone mexicana* L., a road side weed has been selected as the test plant as it is usually exposed to automotive exhausts containing lead contaminants.

Flowers collected from plants growing in the University campus under natural conditions have been the source of pollen for the present experiment. Pollen were germinated in Brewbaker and Kwack's medium[4] at half hour intervals from 4.00 to 7.00 a. m. and optimum germination was found at 6.30 a. m. Pollens were germinated in media containing various concentrations of sucrose and the optimum concentration of sucrose required was determined (Fig. 1). For testing, pollen were germinated in different concentrations of various heavy metal salts added to the Brewbaker's medium containing 12% sucrose (Optimum germination). The length of the pollen tube and the per cent germination (viability) was measured after 15 min using a microscope. Pollen germination was also tested without nutrient medium but with 12% sucrose solution. There were three replicates for each set of pollen grains and the experiment was repeated thrice.

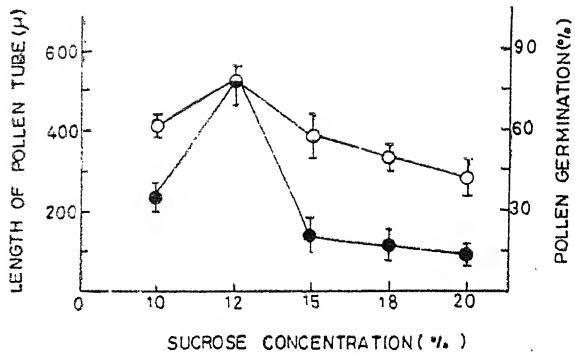

Fig. 1. Pollen response to various concentrations of sucrose in the Brewbaker's medium, 15 minutes after germination.
●—–● Pollen tube length
O----O Per cent pollen germination

Germination of the pollen and the length of the pollen tube was inhibited irrespective of the heavy metal used or sucrose concentration of the medium. All the heavy metals were found to be toxic at higher levels. Pollen tube growth was inhibited even at a low salt concentration of 0.1 ppm. No germination of pollen could be observed above 0.6 ppm of nickel nitrate and above 0.8 ppm of bismuth nitrate. Nickel appears to be most toxic for pollen germination while copper is found to be least toxic (Fig. 2). Bismuth and lead were found to be intermediary in their toxicity. On the basis of our observations following toxicity sequence to pollen germination could be made.

Nickel > Bismuth > Lead > Copper

However, no indepth study was carried out to trace any physiological role of these salts involved in the reduction of pollen tube growth and pollen ge mination. The heavy metals, being toxic, might have disrupted the normal metabolic pathways of germinating pollen. In the present experiment nickel appears to be more toxic as no germination could be observed above 0.6 ppm of nickel and the

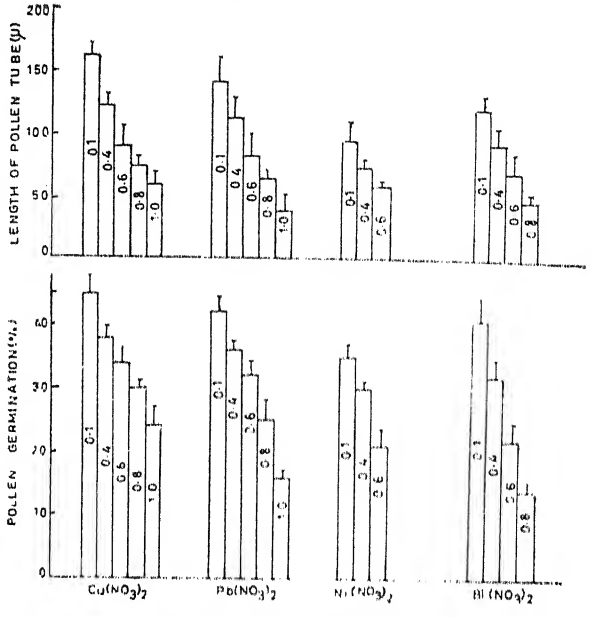

Fig. 2. Pollen germination and length of pollen tube in the presence of various heavy metals at 0.1, 0.4, 0.6, 0.8 and 1.0 ppm concentrations in the medium. Vertical bars indicate standard deviations.

per cent pollen germination and pollen tube growth were also low in 0.1 ppm of nickel as compared to other salts. Nickel was also found to be toxic[2] to germination and root inhibition of rice at a concentration of 5×10^{-4}M.

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Somatic chromosomes of *Apium graveolens* L.

(*Apium graveolens* L./celery/somatic chromosomes/asymmetrical karyotype)

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ABSTRACT Morphology of somatic chromosomes of *Apium graveolens* L. has been studied. Detailed studies on total chromosome length, relative length and arm ratio revealed that karyotype is apparently asymmetrical as most of the chromosomes vary in size and possess subterminal centromeres.

Apium graveolens L., commonly known as celery, is an important essential oil bearing plant which finds extensive use in culinary preparations as well as in perfumery. India is the major producer of this crop and the produce is exported to over 50 countries including USA, USSR, UK, Japan etc. Inspite of being an important commercial crop, no serious attention has so far been paid towards its genetic improvement. A programme in that direction has now been started at this Institute. To start with, an attempt has been made to understand the chromosomal architecture of the species, which is being presented in this note.

Seeds of *Apium graveolens* L. (2n=22) were germinated on thick blotters in a glass petridish at 20±2°C. The roots (1-2mm long) were pre-treated with 0.1% aqueous colchicine solution for 1½ h and fixed in acetic alcohol (1 : 3). They were then stained with aceto-orcein and hydrochloric acid (9 : 1) and squashed in 1% acetocarmine. Camera lucida drawings of the chromosomes were made from temporary slides. Data on total length and arm ratio were taken from 10 well spread metaphase plates. The idiogram was constructed on the basis of the overall mean values of short arm, long arm and total length of 11 homologous pairs (Fig. 3).

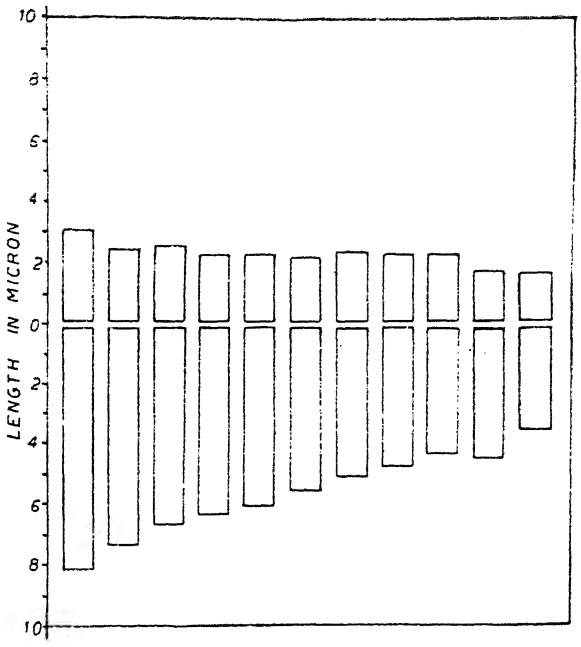

Fig. 3. An idiogram of somatic chromosomes × 1500.

The somatic chromosome number of the species[1] is 2n=22. Present observations confirm these results. Chromosome pairs were numbered from 1 to 11 in order of their decreasing total length. Chromosome 1 was 2.14 times longer than chromosone 11. 11. Chromosome length varied from 11.14 μm for the longest to 5.22 μm for the shortest (Table 1). On the basis of total length the compliment was classified into the following 4 types.

TABLE 1

Morphology of somatic chromosomes of *Apium graveolens* L.

Chromosome number	Total length (micron)	Relative length in proportion to total compliments length (%)	Short arm (micron) S	Long arm (micron) L	Arm ratio S/L
1	2	3	4	5	6
1.	11.14	12.69	3.03	8.11	0.369
2.	9.81	11.18	2.48	7.33	0.338
3.	9.16	10.44	2.54	6.62	0.577
4.	8.66	9.87	2.28	6.38	0.357
5.	8.36	9.52	2.29	6.07	0.377
6.	7.94	9.11	2.18	5.76	0.388
7.	7.50	8.54	2.42	5.08	0.476
8.	7.10	8.09	2.36	4.74	0.360
9.	6.61	7.53	2.33	4.28	0.542
10.	6.24	7.10	1.77	4.47	0.395
11.	5.22	5.96	1.66	3.56	0.466

(A) Three pairs of very long chromosomes ranging in length from 9.16 μm to 11.14 μm.

(B) Three pairs of long chromosomes ranging in length from 7.94 to 8 66 μm.

(C) Three pairs of medium size chromosomes ranging in length from 6.61 to 7.50 μm.

(D) Two pairs of short chromosomes ranging in length 5.22 to 6.24 μm.

On the basis of their arm ratios all the 11 pairs of chromosomes were found to possess subterminal centromeres. The somatic chromosomes (Figs. 1 and 2) and an idiogram of haploid compliments (Fig. 3) standardise the karyomorphology of *Apium graveolens* L.

Karyotype is apparently asymmetrical as most of the chromosomes vary in size and possess subterminal centromeres and falls in category 2B according to the Stebbin's[2] classification.

Thanks are due to Dr. C. K. Atal, Director, Regional Research Laboratory, Jammu for his keen interest and facilities provided for these studies.

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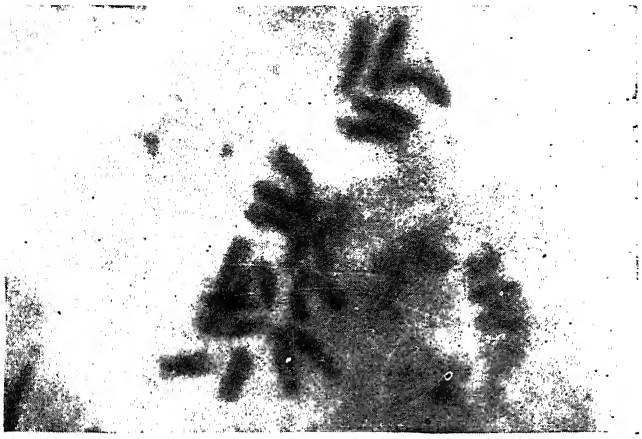

Fig. 1 Microphotograph showing somatic metaphase 2n = 22 × 1500.

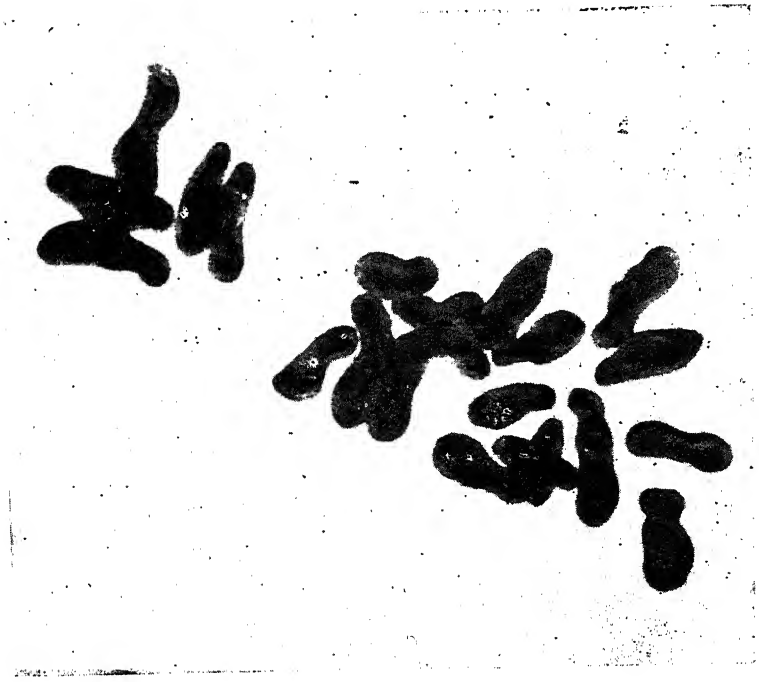

Fig. 2. Camera Lucida drawings of the somatic chromosomes × 1500.

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Luteolin glycoside from the roots of *Sterculia foetida* Linn.

(*Sterculia foetida* Linn./luteolin-7-*O*-β+*D*+galactopyranosyl-(1 → 4)-0-α-L-arabinopyranoside)

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ABSTRACT Luteolin-7-*O*-β-*D*-galacto pyranosyl (1→4,-*O* α-*L*- arabinopyranoside has been isolated from the roots of *Sterculia foetida* Linn.

The bark and leaves of *Sterculia foetida* Linn[1,2] are reported to be aperient and diuretic and used for the treatment of gonorrhoea. Its wood when boiled with seed oil has been found to be useful in rheumatism.

The alcoholic extract of the roots of *Sterculia foetida* was successively extracted with benzene, chloroform, ethyl acetate and methanol soluble fractions.

The ethyl acetate soluble fraction on column chromatography over SiO_2 gave a yellow amorphous mass m.p. 287–88°C. It gave single spot on TLC and crystallised from ethanol in yellow coloured needles, molecular formula $C_{26}H_{28}O_{15}$ λ_{max}^{MeOH} 345, 290sh, 267, 250 nm. ν_{max}^{KBr} 3360 cm^{-1} (-OH group), 2880 cm^{-1} (=CH-stretching), 1740 cm^{-1} (>C=O group), 1660 cm^{-1} (chelated hydroxyl group), 1590, 1150, 1180 cm^{-1} (aromatic nature), 1460 cm^{-1} (-CH-stretching , 810 cm^{-1} (p-substituted phenyl ring). It responded to positive tests for flavonoidal glycoside and when hydrolysed by 7% ethanolic sulphuric acid yielded a light yellow crystalline aglycone m.p. 328°C, molecular formula $C_{15}H_{10}O_6$ and was identified as Luteolin which was further confirmed by m.m.p., Co-TLC and Co-PC with authentic sample[3,4]. The glycoside hydrolysate on paper chromatographic examination showed the presence of *D*-galactose and *L*-arabinose, periodic oxidation of the glycoside confirmed that one molecule of the glycoside was made up of one molecule of the aglycone and one molecule each of *D*-galactose and *D*-arabinose, partial hydrolysis of the glycoside showed the appearance of galactose first followed by arabinose thereby confirming galactose to be the terminal sugar and arabinose to be involved in the glycoside formation. Various UV[5,6] shifts of the glycoside confirmed that C_5-OH group was involved in the glycoside formation. Permethylation of the glycoside followed by hydrolysis yielded 2,3-di-*O*-methyl-α-arabinose and 2,3,4,6-tetra-*O*-methyl-*D*-galactose thereby confirming that C_1 of the *D*-galactose was linked to $C_4$of *L*-arabinose and further confirmed that both sugars were present in the pyranose form. The glycoside was thus assigned the structure as Luteolin-7-*O*-β-*D*-galactopyranosyl-(1→4)-*O*-α-*L*-arabinopyranoside.

One of us (A.S.) thanks C.S.I R., New Delhi, for financial assistance.

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A non-ionic seed-gum from *Crotalaria mucronata* of Indian origin

(*Crotalaria mucronata*/galactomannan/oligosaccharides)

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ABSTRACT A water-soluble D-galacto-D-mannan has been isolated from the seeds of *C. mucronata* of Indian origin, containing D-galactose and D-mannose in 3 : 7 molar ratio. Acid catalysed fragmentation, periodate oxidation, methylation and enzymic hydrolysis showed that the seed-gum has a branched structure consisting of a linear chain of β-D-(1→4) linked mannopyranosyl units, some of which are substituted at 0-6 by α-D-galactopyranosyl units, glycosidically.

Majority of plants of Crotalaria[1] genus are reputed for their therapeutic value. Recently it is reported that *C. mucronata* and *C. saltiana* are synonyms. The plant *C. mucronata*[2] is a native of Africa but cultivated in other continents also. Unrau and Choy[3] chemically examined the seed-gum from *C. mucronata* and reported a galactomannan where the ratio of the two building monosaccharides (23 : 70) was found quite different from ours (3 : 7). These differences may arise due to ecological variations in different natural sources of their availability. Sometimes minor differences creep in due to the analytical procedures followed.

Polysaccharide was conveniently extracted from the crushed, defatted and decolourised seeds by extraction with 1% aqueous acetic acid and by repeated precipitation from its solution therein with ethanol. It was purified, and tested for homogeneity by usual methods. The white amorphous polysaccharide had $[\alpha]_D^{25}$ + 68° (water), an ash content of 0.3%, and a negligible percentage of methoxyl, acetyl and uronic acid contents. Upon complete acid-hydrolysis the polysaccharide yielded *D*-galactose and *D*-mannose in 3 : 7 molar ratio. Graded acid hydrolysis resulted in the preferential removal of α–linked *D*-galactose units on the periphery as end-groups. To determine the position of linkages between the building units of the galactomannan, it was exhaustively methylated by Haworth–Purdie method, to afford a brown, semisolid glassy mass and had $[\alpha]_D^{28}$ + 41° (chloroform). Hydrolysis of the methylated seed-gum gave 2, 3, 4, 6-tetra-*O*-methyl-*D*-galactose (3 mol.), 2, 3, 6-tri-*O*-methyl-*D*-mannose (4 mol.) and 2, 3,-di-*O*-methyl-*D*-mannose (3 mol.). The identity of these methylated monosaccharides was established on the basis of their R_{TMG} values, optical rotations and crystalline derivatives. The percentage of end groups calculated from methylation studies was 29.9%. Oxidaion of the seed-gum with sodium metaperiodate consumed 845 mM of the oxidant with the liberation of 183 mM of formic acid per 100 g of the poly saccharide indicating 29.5% end-groups (*cf.* methylation).

Acid catalysed partial hydrolysis of the seed-gum gave, two disaccharides : α–*D*-Galp (1→6)-*D*-Manp,

β-*D*-Manp (1 → 4) -*D*-Manp and two trisaccharides α-*D*-Galp (1 → 6) -β-*D*-Manp (1 → 4) -*D*-Manp and β-*D*-Manp (1 → 4) -β-*D*-Manp (1 → 4)-β-*D*-Manp alongwith the component sugars. All of the oligosaccharides were characterised. These results corroborated the earlier findings. The foregoing data accord with the following structure.

α *D* Galp
1
↓
6
→ 4) β *D* Manp (1[→ 4) β *D* Manp (1]$_2$→ 4)

α *D* Galp 1 ↓ 6 β *D* Manp (1 [→4) β *D* Manp (1]$_2$→4) β *D* Manp(1 → α *D* Galp 1 ↓ 6

Paper chromatography was conducted on whatman No. 1 and 3 MM papers by descending technique using the following systems v/v.

A- 1-Butanol-ethanol-water (5 : 1 : 4)[4], B- 1-Butanol-isopropanol-water (11 : 6 : 3)[5], C- Ethylacetate-pyridine-water (2 : 1 : 2)[5].

Solutions were concentrated at diminished pressure and at low temperature. All residues were dried in vaccuo over anhydrous calcium chloride, melting points are uncorrected and $[\alpha]_D$ values are for equilibria.

Dried and crushed seeds (1 kg) were successively extracted with light petroleum and ethanol to remove fatty and colouring substances respectively. Polysaccharide was extracted with 1% aqueous acetic acid and precipitated with 90% ethanol. It was purified by repeated deproteinization[7] with chloroform and complexation with Fehling's solution[8]. Homogeneity was tested by fractional precipitation[9], zone-electrophoresis[10] and *via* acetylation[11] and deacetylation[11]. The pure polysaccharide was hydrolysed with 2N sulphuric acid, and the hydrolysate was fractionated by p.c. (solvent-B) on preparative scale. Quantification[12] by periodate oxidation showed the molar ratio of 3 : 7 between *D*-galactose and *D*-mannose.

Graded hydrolysis[13] with 25 mM sulphuric acid (monitored by p.c., solvent-B) resulted in the favoured removal of *D*-galactose (20 min), followed by *D*-mannose (30 min).

Polysaccharide was exhaustively methylated first by Haworth's method[14] and then by Purdies method[15]. Completely methylated polysaccharide $[\alpha]_D^{28}$ + 41° (chloroform) was hydrolysed[16] and the hydrolysate was fractionated (p.c., solvent-A) on preparative scale using Whatman No. 3 MM paper. The resulting methylated sugars were quantified by alkaline hypoiodite method[17].

1. 2, 3, 4, 6-*tetra-O-methyl-D-galactose*[18] (3 mol.) : R_{TMG} (solvent-A), 0.87, m p. 72–73°, $[\alpha]_D^{32}$ + 120° (water). The anilide had m.p. 192–193°, $[\alpha]_D^{32}$ + 43° (acetone).
2. 2, 3-*di-O-methyl-D-mannose*[18,19] (3 mol) : R_{TMG} (solvant-A), 0.52, syrup, $[\alpha]_D^{28}$-14° (water). The anilide had m.p 136°.
3. 2, 3, 6-*tri-O-methyl-D-mannose*[20,21] (4 mol) : R_{TMG} (solvent-A), 0.80, syrup, $[\alpha]_D^{28}$ - 11°. The derived phenylhydrazide had m.p. 130°.

Polysaccharide (300 mg) was oxidised with 25 ml of 0.25 M sodium metaperiodate by the method of Andrews *et al.*[22] 845 mM of periodate were consumed with the simultaneous liberation of 183 mM of formic acid per 100 g of the polysaccharide.

The polysaccharide (6.0 g) was partially hydrolysed with 50 mM sulphuric acid for 12 h at 100° and the hydrolysate was examined by p.c. (solvent C). Following oligosaccharides were detected. These oligosaccharides were separated and identified

1. *Epimelibiose* : α-*D*-Galp (1 – 6)-*D*-Manp[6,23] : m.p. 200°, $[\alpha]_D^{32}$ + 120° (water), phenyl osazone m.p 173°, acid hydrolysis gave p.c. (solvent-B) galactose and mannose, and methylation followed by hydrolysis gave p.c. (solvent-A), 2, 3, 4, 6-tetra-*O*-methyl galactose and 2, 3, 4-tri-*O*-methyl-*D*-mannose.

2. *Mannobiose* : β-*D*-Manp (1 – 4)-*D*-Manp[24,25] ; m.p. 203–205° (from ethanol), $[\alpha]_D^{25}$ – 9° (water). The derived phenylosazone had m.p.[25] 204°. Acid hydrolysis gave mannose only p.c. (solvent-B) and emulsin hydrolysed the disaccharide, indicating a β-linkage. Methylation followed by hydrolysis gave p c (solvent–A) 2, 3, 4, 6-tetra-*O*-methyl, and 2, 3, 6-tri-*O*-methyl-*D*-mannose.
3. *Mannotriose* : β-*D*-Manp (1 – 4) – β-*D* – Manp (1→4) *D*-Manp[26] : m.p. 161–163° (from ethanol), $[\alpha]_D^{25}$ – 23° (water) acid hydrolysis gave p.c. (solvent–B) mannose only, and partial hydrolysis with acid gave p c. (solvent–C) mannobiose and mannose. Methylation followed by hydrolysis gave p.c. (solvent–A) 2, 3, 4, 6-tetra- and 2, 3, 6-tri-*O*-methyl-*D*-mannose. The trisaccharide was cleaved by emulsin, showing intersugar linkages as β.
4. *Galactosyl mannobiose* : α-*D*-Galp (1 – 6)-β-*D*-Manp (1→4)-*D*-Manp[23] : m.p. 227° $[\alpha]_D^{25}$ + 92–93° (water). Partial hydrolysis gave p.c. (solvent–C) mannobiose, epimelibiose, galactose and mannose. During 48 h the trisaccharide consumed 6.02 mol of sodium metaperiodate and liberated 2.98 mol of formic acid.

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Spectrophotometric studies of mechanism of oxidation of 2,2'-dihydroxy diethyl ether by osmium tetroxide

(kinetics/oxidation mechanism/OsO_4/2,2'-dihydroxy diethyl ether)

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ABSTRACT Spectrophotometric studies of kinetics of osmium tetroxide oxidation of 2, 2'-dihydroxy diethyl ether (2, 2'-DDE) in alkaline media have been studied. The reaction follows simple first order kinetics with respect to osmium tetroxide and 2, 2'-DDE as well. First order dependence of the reaction at low [OH^-] tends to zero order at its higher concentrations. The reaction remains unaffected by ionic strength variation. Activation parameters are reported. The suggested mechanism refers only to the initial stage of the reaction.

Earlier Os(VIII) has been widely used as a catalyst[1-5] in oxidation of various substances as well as an oxidant[6-13]. As a part of our broad program, kinetics and mechanism of Os(VIII) oxidation of 2, 2'-DDE in alkaline media is reported in the present communication.

The solution of OsO_4 (Johnson and Matthey) was prepared by dissolving its one g in 393.4 ml of KOH solution (0.05M). B.D.H. (A.R.) sample of 2,2'-DDE and all other reagents of A.R. grade were used. Triple distilled water was employed in all experiments.

The reaction was started by the method described by other workers[14-16]. Osmate ion absorbs strongly in the visible region 400 nm ($\epsilon = 400$). The kinetics were followed by spectrophotometric analysis of remaining [osmium tetroxide].

Spectrophotometric analysis of unconsumed [OsO_4] in different sets with varying oxidant-reductant ratios indicated consumption of two moles of OsO_4 for the oxidation of per mol of 2, 2'-DDE. Accordingly, stoichiometric equation may be written as given below.

$$\begin{matrix} CH_2OHCH_2 \\ CH_2OHCH_2 \end{matrix} \diagdown\!\diagup O + 2\,OsO_4 + 4OH^- + H_2O = 2[OsO_2(OH)_4]^{2-} + 2\,CH_3COOH$$

Data in Tables 1 and 2 show first order dependence of the reaction rate on [OsO_4] and [2, 2'-DDE], respectively. Data of Table 3 show the effect of variation of [OH^-] on reaction rate. The values of k_2 in 3rd column of Table 3 remain constant at low [OH^-] but decrease on increasing[OH^-] which shows the tendency of the reaction to shift from first order at low (OH^-) to zero order at higher (OH^-). The effect of ionic strength maintained by adding KNO_3 is negligible. The reaction was studied in the temperature range 27-40°C. Activation energy and entropy were computed and found as 51.00 k J mol^{-1} and 90.66 J K^{-1}, respectively.

It is assumed that in alkaline medium there is no Os(VIII) in the form of OsO_4, which is confirmed by the formation[17] of $[OsO_4(OH)_2]^{2-}$ with OH^- ions. It has been reported that osmium tetroxide gives octahedral complexes[18] of the form [OsO_4(OH)

TABLE 1

Effect of varying $[OsO_4]$ on reaction rate at 35°C
[2,2,'-DDE] = 10.00×10^{-2}M, [NaOH] = 2.00×10^{-2}M

$[OsO_4] \times 10^3$M	1.00	1.34	1.67	2.00	2.50	3.34	4.00
$k_{obs} \times 10^4$ s^{-1}	8.01	8.21	8.13	8.53	8.40	8.01	8.23

TABLE 2

Effect of varying [2, 2'-DDE] on reaction rate at 36°C
$[OsO_4] = 1.00 \times 10^{-3}$M

[2, 2'-DDE] × 10M	$k_{obs} \times 10^4$ s^{-1}		$k_2 \times 10^2$ l mole^{-1} s^{-1}	
	*	**	*	**
0.34	2.88	1.92	0.86	0.59
0.67	5.71	3.97	0.86	0.59
1.25	11.24	7.84	0.90	0.62
2.50	22.64	15.99	0.91	0.63
3.34	27.35	19.11	0.82	0.57
5.00	39.44	29.80	0.80	0.60

*[NaOH] = 2.00×10^{-2}M and **[NaOH] = 1.25×10^{-2}M

TABLE 3

Effect of varying $[OH^-]$ on reaction rate at 34°C
$[OsO_4] = 1.00 \times 10^{-3}$M

[NaOH] × 10^2M	$k_{obs} \times 10^4$ s^{-1}		$k_2 \times 10$ l mol^{-1} s^{-1}	
	*	**	*	**
1.20	3.36	3.84	0.28	0.32
2.50	6.95	8.09	0.28	0.32
5.00	13.16	15.50	0.26	0.31
6.67	16.50	20.01	0.25	0.30
10.00	17.80	23.22	0.18	0.23
13.34	22.50	26.77	0.17	0.20
20.00	28.33	32.61	0.14	0.16

*[2, 2'-DDE] = 10.00×10^{-2}M and **[2, 2'-DDE] = 12.50×10^{-2}M

$(H_2O)]^{1-}$ and trans- $[OsO_4(OH)_2]^{2-}$. The former species is involved in step (2) of the proposed scheme for the reaction. It may be noted that $[OsO_4(OH)_2]^{2-}$ is the only reactive species formed by osmium tetroxide and the equilibrium lies entirely in its favour[19].

In the light of these observations, the oxidation mechanism proposed is given by step (1-4) where S represents 2, 2'-dihydroxy diethyl ether (2, 2'-DDE).

$$S + OH^- \overset{K}{\rightleftharpoons} S^- + H_2O \quad (1)$$

$$[OsO_4(OH)(H_2O)]^{1-} + OH^- \overset{K_1}{\rightleftharpoons} [OsO_4(OH)_2]^{2-} + H_2O \quad (2)$$
$$(C_1) \qquad\qquad (C_2)$$

$$S^- + [OsO_4(OH)_2]^{2-} \overset{K_2}{\rightleftharpoons} \underset{(C_3)}{X\ (\text{Intermediate})} \quad (3)$$

$$X \xrightarrow[\text{slow}]{k_1} [OsO_2(OH)_4]^{2-} + Y \text{ (another intermediate)} \quad (4)$$

$$Y + [OsO_4(OH)_2]^{2-} \xrightarrow[\text{fast}]{k_2} \text{products} \quad (5)$$

Scheme 1

Formation of $[OsO_2(OH)_4]^{2-}$ in step (4) is supported by Cotton and Wilkinson[17] who have shown that Os(VI) exists as $[OsO_2(OH)_4]^{2-}$.

Total [Os(VIII)] may be obtained by eqn (6) from above steps.

$$[Os(VIII)]_T = [C_1] + [C_2] + [C_3] \quad (6)$$

The final rate, derived on the basis of consideration of equilibrium conditions for steps (1-3) and total [Os(VIII)] from eqn (6) may be given by eqn (7) with reasonable assumption[1] $K_1[OH^-] \gg 1$

$$-\frac{d[OsO_4]}{dt} = \frac{2\,k_1\,KK_2\,[Os(VIII)]_T\,[S]\,[OH^-]}{1 + KK_2\,[S]\,[OH^-]} \quad (7)$$

Eqn (7) may be written as

$$\frac{1}{\text{rate}} = 1/\left(-\frac{d\,[OsO_4]}{dt}\right) =$$

$$\frac{1}{2k_1\,KK_2\,[Os(VIII)]_T\,[S]\,[OH^-]} + \frac{1}{2k_1\,[Os(VIII)_T]} \quad (8)$$

A plot between 1/rate and $1/[OH^-]$ gave a straight line with an intercept on 1/rate axis. The value of $k_1\,KK_2$ was calculated from the slope of the straight line and was found to be 1.40×10^2. At low $[OH^-]$,

the inequality $KK_2[S][OH^-] \ll 1$ exists and eqn (7) is reduced to

$$-\frac{d[OsO_4]}{dt} = 2\, k_1 KK_2 [Os(VIII)]_T\, [S]\, [OH^-] \quad (9)$$

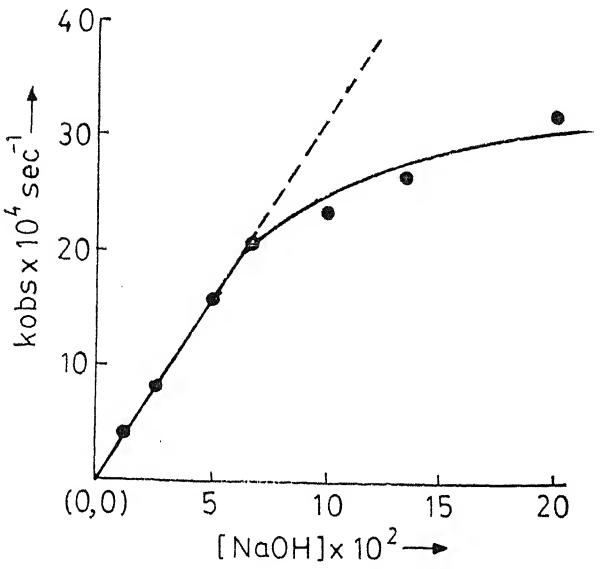

Fig. 1 Under the conditions of Table 3.

The value of $k_1 KK_2$ was also calculated from eqn (9) and was found to be 1.36×10^2. Thus $k_1 KK_2$ values obtained from eqn (8) and (9) are quite close to each other which justifies the mechanism proposed by us. At higher $[OH^-]$, the inequality $KK_2\,[S]\,[OH^-] \gg 1$ tends to exist which shows the tendency of the reaction rate to decrease on increasing $[OH^-]$ as is obvious from Fig. 1 and reaction rate tends to become independent of $[OH^-]$ in the higher range. The value of k_2 (Table 3) which was nearly constant in the lower range of $[OH^-]$ also decreases in the higher range of [OH], which is indicative of change of order from first observed at lower range of $[OH^-]$. to zero in higher range of $[OH^-]$. The suggested mechanism is only known to apply during initial period and could be different in later stages.

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Copper(II) complexes with Schiff bases

(Schiff base/copper complexes/magnetic moment

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ABSTRACT The copper(II) complexes of Schiff bases derived from substituted benzaldehyde and differently substituted aromatic amines were synthesised and characterised by elemental analysis, ir, uv, visible and diffused electronic reflectance spectra and magnetic susceptibility measurements. Most of the complexes exhibit normal magnetic moments at room temperature barring few cases where magnetic moment is subnormal indicating Cu Cu interaction.

Schiff bases derived from 2-hydroxy aromatic aldehydes and chloroaniline or with mercapto group can function as bidentate, tridentate and bridging type ligands in their complexes with metal ions. Copper complexes of these Schiff bases have an interesting possibility of structural variations depending on the involvement of the chloro, hydroxy or mercapto group of the amine moiety and counter anion in co-ordination. In continuation of our previous work[1,2] on synthesis of copper complexes of differently substituted Schiff bases, the effect of and chelating properties of different functional groups on either phenolic ring on Schiff base is studied here. The copper complexes derived from these Schiff bases were characterised by their elemental analysis, electronic, uv, and ir spectra and magnetic susceptibility measurements.

The Schiff bases were prepared by condensing stoichiometric proportions of substituted aldehyde with substituted amine. In the present work Schiff bases derived from : (1) 2-hydroxy benzaldehyde and 2-hydroxy aniline (A_1), 2-hydroxy-5-chloro aniline (A_2), 2-mercapto aniline (A_3), (2) 2-hydroxy-5-nitro benzaldehyde and 2-hydroxy aniline (B_1), 2-hydroxy-chloro aniline (B_2), 2-mercapto aniline (B_3), (3) 3-nitro benzaldehyde and 2-hydroxy aniline (C_1), 2-hydroxy-5-chloro aniline (C_2), (4) 2-hydroxy - 3 - methoxy benzaldehyde and 2-hydroxy aniline (D_1), 2-hydroxy-5-chloro aniline (D_2), 2-mercapto aniline (D_3) were synthesised. The refluxion was carried out for about 30 min. The solid obtained was filtered at suction and dried in air. After crystallising it from their alcoholic solution, where permitted by solubility, they were characterised by their elemental analysis and melting point.

Copper complexes of the above Schiff bases were prepared by mixing Cu(II) (1 mg/ml) solution with 1% alcoholic solution of the respective Schiff base in 1 : 1 proportion at optimum pH, at which corresponding metal complex was precipitated. The optimum pH of precipitation was decided by carrying out precipitation at different pH and the pH at which maximum yield was obtained was considered as optimum pH. The precipitate was digested on water bath for 30 min. and then filtered, washed with deionised water and dried at 105°C till constant weight. These complexes were characterised on the basis of their elemental analysis, magnetic susceptibility, uv, visible, ir and diffused reflectance electro-

nic spectral studies. Beckman DU 2 Spectrophotometer was used to record uv, visible spectra, while diffused reflectance electronic spectra were recorded on Carl-Ziess VSU2P Spectrophotometer using MgO as a reference and diluent. The ir spectra were recorded on Higher-Watt Spectrophotometer and Guoy balance method was used to carry out magnetic susceptibility measurements. These data are tabulated in Table 1.

complexes like CuB_2 and CuD_2 where it is in the acidic pH. These complexes are stable upto 110–120°C, beyond which probably they start decomposing as indicated by weight loss during the isothermal heating experiments.

The subnormal room temperature magnetic moment shown by the complexes, except CuB_2, CuB_3 and CuD_1 is an indication of Cu-Cu interaction in the solid state. A dimetric structure such that

TABLE 1

Analytical and physical data of Cu complexes

Compound	Elemental Analysis				pH range	Colour	Thermal stability (°C)	Absorption bands in solution (nm)	d-d band (kK)	μ_{eff} (B.M)
	C%	H%	N%	Cu%						
$Cu-A_1$	57.71 (56.62)	4.03 (3.99)	5.4 (5.08)	22.2 (23.06)	5.0–6.0	Green	120	225,420	13.5	1.40
$Cu-A_2$	48.91 (50.32)	3.22 (3.23)	4.44 (4.52)	21.05 (20.49)	7.0–8.0	Yellowish green	120	227.428	14.3,12.2	1.68
$Cu-A_3$	53.87 (53.51)	3.85 (3.77)	5.73 (4.80)	20.18 (21.81)	7.0–8.0	Greenish brown	120	234,288,328,390	15.5,14.3,12.2	1.66
$Cu-B_1$	49.12 (48.66)	3.77 (3.11)	8.87 (8.73)	19.06 (19.82)	5.0–6.0	Green	120	270,420	16.2,13.8	1.46
$Cu-B_2$	44 82 (43.88)	1.90 (2.53)	7.39 (7.86)	16.77 (17.87)	4.0–5.0	Green	100	282,420,430	15.2	1.74
$Cu-B_3$	46.78 (45 22)	1.79 (2.96)	7.88 (8.30)	17.44 (18.24)	6.0–7.0	Greenish brown	120	228,288,365,400	14.9,11.4,12.2	1.88
$Cu-C_1$	51.22 (50.70)	3.28 (3.11)	9.19 (8.75)	20.80 (21.15)	7.5–8.5	Brown	110	350,370	16.7,11.6,11.1	1.00
$Cu-C_2$	46.86 (46.01)	3.33 (2.65)	6.81 (8.26)	17.66 (18.74)	8.0–10.0	Brown	110	245,425	13.9	1.72
$Cu-D_1$	54.90 (56.84)	3.93 (4.40)	4.58 (4.75)	20.90 (20.90)	6.5–7.5	Green	100	310,428	16.1,~10.6	1.83
$Cu-D_2$	49 10 (49.41)	4.19 (3.53)	4.60 (4.12)	17.50 (18.69)	3.0–4.0	Yellowochre	120	235,435	15.6,13.5	1.69
$Cu-D_3$	50.59 (52.25)	3.30 (4.04)	4.01 (4.35)	18.93 (19.76)	7.0–8.0	Moss green	110	304	14.3	1.58

The critical examination of data given in Table 1 reveals that copper combines with Schiff base by replacing two protons from the ligand. except in case of CuC_1 and CuC_2. The 1 : 1 stoichiometry is observed for metal : ligand, The optimum pH for precipitation of complexes is around 7.0, barring few

significant spin exchange between the two copper ions could occur is suggested. Such interaction can take place either directly or through oxygen bridges. It has been found that the Schiff base derived from 3 - nitro benzaldehyde and 2 - mercapto aniline, which does not contain phenolic - OH, fail to give

1 : 1 copper complexes. This observation further supports the above suggestion. Conclusively the results of magnetic measurements are typical of Cu(II) complexes containing tri co-ordinate conjugate ligand units, which are reported[3] to be planar.

Table 1 shows λ_{max} for the uv-visible absorption bands in methanolic solution, except for CuD_3 which is in DMF. These are the modified intra ligand $n \rightarrow \pi^*$ transition band with bathochromic shifts in most cases. The complexes do not show the absorption bands at 350 – 370 nm which are present in the spectra of ligands and are characteristic of phenolic compounds. This observation further indicates that the metal-ligand bonding takes place through oxygen atom of phenolic groups. The bands in the visible region (400 nm) are probably due to the presence of chromophoric substituents in the aromatic ring. The diffused reflectance electronic spectra reveal *d-d* bands in the region 13.5 – 16.7 kK. These are also given in Table 1. These bands are in the range, expected for distorted octahedral, virtually planar copper(II) complexes. The broadness and structure of bands is characteristic of distortion from cubic geometry. The observed frequency of the *d-d* bands gives qualitatively the relative strength of ligand fields around Cu(II). Accordingly, the ligands studied in the present work may be arranged as $C_1 > B_1 \sim D_1 > A_3 \sim D_2 > B_2 > B_3 > A_2 \sim D_3 > C_2 > A_1$.

The spectra of Schiff base ligand broad to medium intensity stretching vibrations in the region 3400 – 3050 cm^{-1}, due to very strong hydrogen bonding present in the solid state. However, these bands are absent in the spectra of their corresponding copper complexes, which indicates that the metal ligand co-ordination takes place through phenolic group The weak intensity of these bands in case of ligand C_1 and C_2, is probably due to only one phenolic group in the ligand, as compared with the other ligand studied. A weak intensity - SH stretching vibration is observed in case of ligand A_3 and D_3 around 2500-2425 cm^{-1}. This band also disappears on complex formation with Cu(II), indicating involvement of S atom of mercapto group. The stretching vibration due to ν C=N is observed in ligands and their complexes in the range 1665-1600 cm^{-1} with strong to medium intensity. This ν C=N band is found to be lower than in the spectra of the corresponding free ligand, showing that the copper(II) binds through the azomethine group of the Schiff bases. The characteristic nitro group asymmetric stretching modes with a strong intensity are located at 1555-1525 cm^{-1}, which are also found in complexes in the same range. Also symmetric stretching vibrations of the $-NO_2$ group appear in range 1355-1290 cm^{-1} as strong to medium intensity bands. The bending mode due to phenolic - OH appears in the range 1415-1360 cm^{-1}, with medium to weak intensity, in the spectra of ligands. The fact that, this band is absent in the spectra of corresponding copper(II) complexes, further suggests that the O of the phenolic OH binds to Cu(II). The stretching band ν C – O are observed in the range 1215-1150 cm^{-1} and are found to be shifted to lower frequencies as expected on bonding to Cu(II) through O atom.

On critical examination of the spectra of A_2, B_2, C_2, D_2 and their corresponding Cu(II) complexes specially at lower frequencies, the complexes show some additional bands of strong intensity around 750-685 cm^{-1} which are assigned to stretching modes of ν C - Cl aromatic vibrations. Similarly, spectra of A_3, B_3, D_3 and their Cu(II) complexes show some weak absorption bands around 850-795 cm^{-1} due to ν C – S. All the assignments to ir bands have been made after accounting for the bands due to aromatic ring vibrations.

From the above arguments it is seen that the Cu(II) complexes of the Schiff base studied in the present work possess distorted octahedral geometry,

which may be planar. Also the co-ordinating properties of various functional group on Schiff bases studied,do show some variations in some of the physical characteristics of the Cu(II) complexes, but overall structure remains some. The ligand field strength of differently substituted Schiff base can be correlated with each other and the results can be utilised for the analytical aspects in determination of copper.

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Optical behaviour of a mixture of mesogenic and non-mesogenic molecules

(liquid crystals/optical studies/mesogenic and non-mesogenic mixtures)

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ABSTRACT Measurements of refractive indices and birefringence have been made on technologically important mesogen E43 and in its mixtures with two non-mesogenic compounds, one highly polar and the other weakly polar. The highly polar solute molecule decreases n_e to a larger amount in comparison with weakly polar solute molecule. For larger concentrations of the solute there appears a two phase co-existence region. The width of the two phase region is larger (~16°C) in the mixture of weakly polar solute than with highly polar solute (~8°C). In this region the birefringence $\triangle n$ and the order parameter (S) fall more rapidly with rising temperature than in their nematic phases, in contrast to that observed for nematic binary mixtures where they remain constant.

The physical properties of a pure nematogen can be significantly changed if a second component is added. There have been several studies[1-5] on mixtures of mesomorphic compounds for their relative concentrations. However, there has been relatively little study[6,7] in mixtures where one of the compounds is non-mesogenic. The present study is reported on the optical behaviour of mixtures of a mesogen and non-mesogens (highly and weakly polars) at different concentrations. It is expected to throw light on the behaviour of refractive indices, order parameter etc. of the mixtures and to identify the formation of two-phase co-existence region.

Measurements of birefringence ($\triangle n = n_e - n_o$), ordinary refractive index (n_o) and transition temperature T_{NI} have been made on technologically important mesogen E43 and its mixtures with two non mesogenic molecules : toluene and benzonitrile. The studies were conducted at 3 and 7.5% concentrations (in molar percentage) of the non-mesogenic solute.

The ordinary (n_0) and isotropic (n) refractive indices were measured using an Abbe Refractometer (accuracy. 001) at 589.6 nm (D_1 sodium line). The prisms were coated with 1% alkaline solution of PVA in benzene to induce homogenous alignment.

The nematic-isotropic transition temperatures were determined using a polarizing microscope fitted with a hot stage arrangement.

The birefringence $\triangle n$ was measured using the wedge technique[8]. The technique essentially consists of measuring the birefringence separation $\triangle X$ (transmission geometry) in the gap (filled with liquid crystal material) of the wedge formed by the two glass plates. $\triangle n$ is then given by

$$\triangle n = \frac{\lambda l}{d \triangle X} \qquad (1)$$

where d is the width of the mylar sheet and l is the

distance of the mylar sheet from the edge of the wedge.

Throughout the experiment temperature was measured by using a calibrated constantan-copper thermo couple and the accuracy of temperature measurement was $\pm .1°C$.

The refractive indices have been analyzed following the method of Haller[9] and Horn[10] to give the order parameter (S) of the nematic phase. This method uses Vuks[11] relation.

$$S(\triangle \alpha/\alpha) = \frac{3(n_e^2 - n_o^2)}{[n_e^2 + 2n_o^2 - 3]} \qquad (2)$$

where $\triangle \alpha$ is the polarizability anisotropy and α is the mean molecular polarizability.

The extrapolation of $S(\triangle \alpha/\alpha)$ to $T = 0K$ (where $S=L$) yields $\triangle \alpha/\alpha$. Inserting this value of $\triangle \alpha/\alpha$ in eqn. (2) gives S.

The ordinary refractive indices of E43, E43+$C_6H_5CH_3$ (3%) and E43+C_6H_5CN (3%) are shown in Fig. 1, as functions of reduced temperature (T/T_c). The ordinary refractive index (n_o) changes only slightly with the addition of highly and weakly polar non-mesogenic compounds, the effect is noticeable only close to the clearing point where n_o exhibits a sudden first order change at the nematic isotropic transition temperature. The extra-ordinary (n_e) as well as isotropic (n) refractive indices decrease with rise in temperature in all cases, but the effect with n_e is larger. The effect of a highly polar non-mesogenic compound (benzonitrile) to reduce n_e is much greater than that of a weakly polar toluene. The variation of order parameter of E43 and different mixtures is shown in Fig. 2. The values of order parameter and refractive indices for E43 are consistent with those reported earlier[12]. From the figure it appears that the highly polar solute slightly increases the order parameter of the mesogen, whereas the weakly polar solute causes a disordering in the molecular alignment. Also the value of order parameter lies in the range : 0.75 – 0.40, i.e. the mesophase stability decreases with increasing temperature. This behaviour of the variation of refractive indices and order parameter is similar to that observed in mixtures of two mesogens[4].

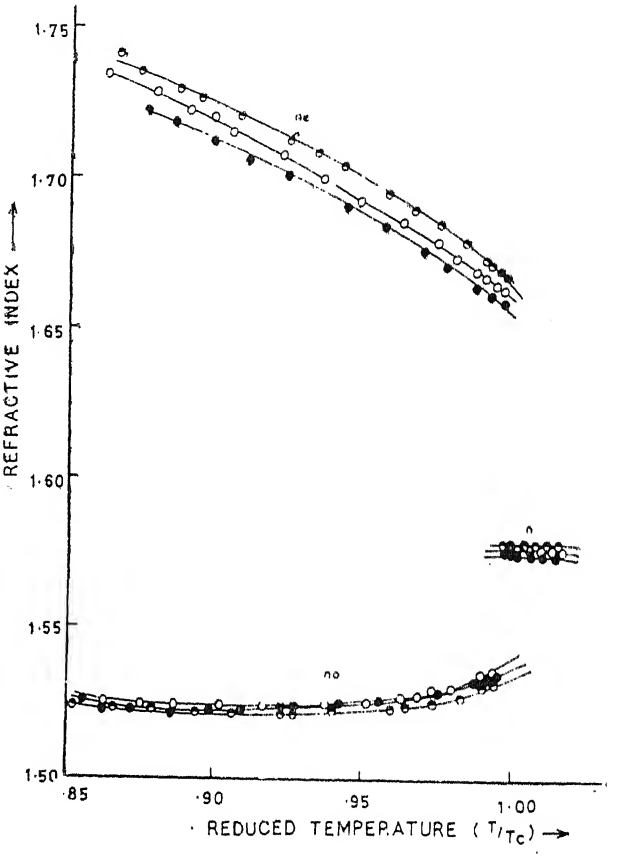

Fig. 1. Temperature variation of the refractive indices (n_e, n_o and n) of E43 – ○–○–○–, E43+$C_6H_5CH_3$ (3%) –o–o–o– and E43+C_6H_5CN (3%) –●–o–●–

The behaviours of n_o, $\triangle n$ and S for the mixtures of E43 with 7.5% concentrations of benzonitrile and toluene are shown in Fig. (3 & 4). With benzonitrile mixture n_o increases slowly with rise in temperature and at 67°C, it exhibits a sudden jump. After this it decreases slowly and at 75.5°C nematic-isotropic transition is obtained. Similar behaviour of n_o, with transitions at 69 and 85.5°C, has been observed

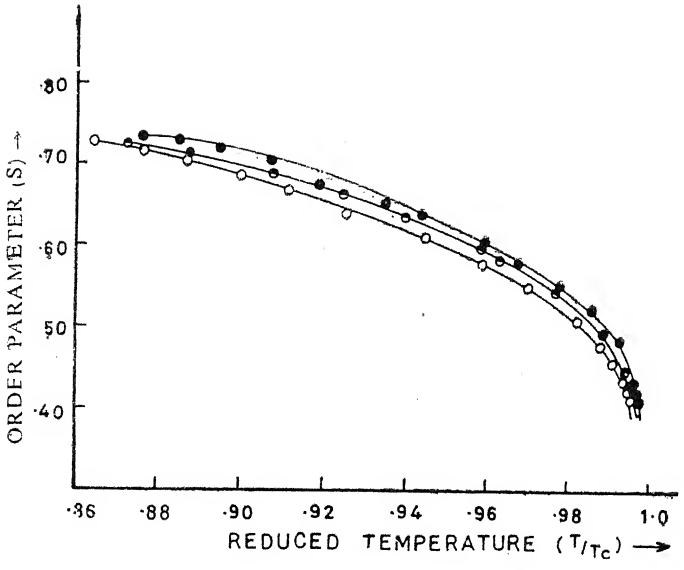

Fig. 2. Temperature variation of the order parameter (S) of E43 -◒-◒-◒-, E43 + $C_6H_5CH_3$ (3%)-o-o-o- and E43 + C_6H_5CN (3%) -● ●-●-

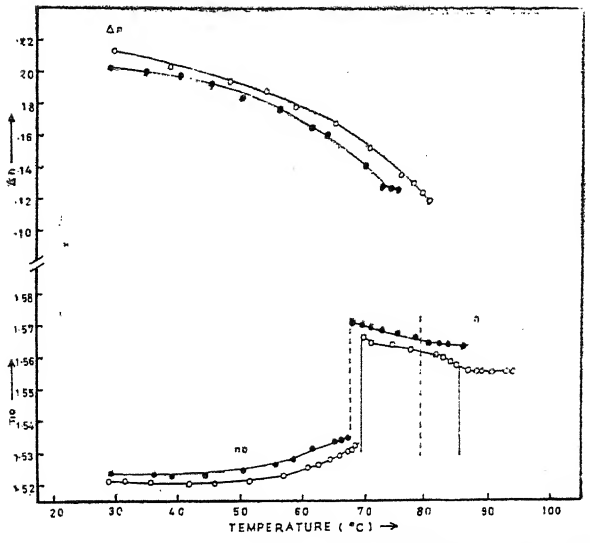

Fig. 3. Temperature variation of the refractive indices (n_o and n) and birefringence (Δn) of E43 + $C_6H_5CH_3$ (7.5%) -o-o-o- and E43 + C_6H_5CN (7.5%) -●-●-●-

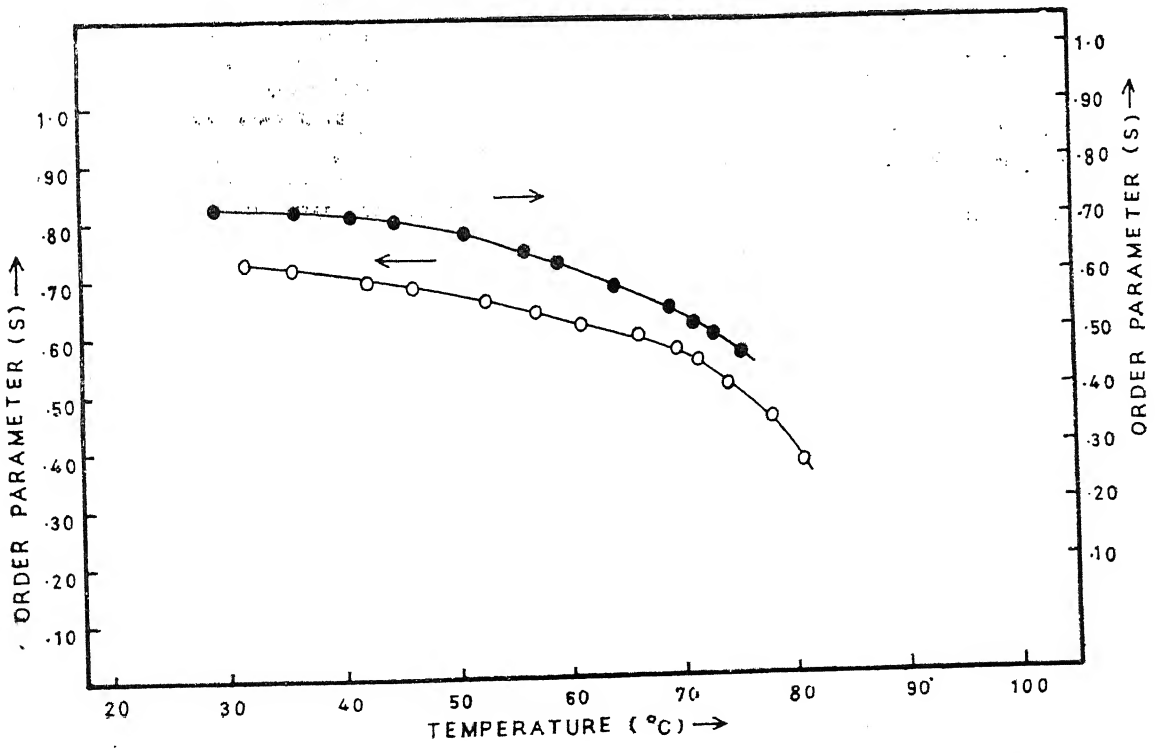

Fig. 4 Temperature variation of the order parameter of E43 + $C_6H_5CH_3$ (7.5%) -o-o-o- and E43 + C_6H_5CN (7.5%) -●-●-●-

with toluene mixture. In the temperature range between the two transitions, there appears a two-phase co-existence region. These transition temperatures were also observed using polarizing microscope fitted with hot stage. The phenomena of two-phase co-existence region in mixtures of mesogens have been observed by many workers[2,3] and it occurs close to the phase transition in a narrow temperature range[2] (not more than 2.5°C). Palffy-Muharay *et al.*[3] reported that Maier-Saupe mean field theory may be applied to systems containing two mesogens or a mesogen and a non-mesogen. The extent and shape of the two-phase co-existence region depend on the ratios of the nematic-isotropic transition temperatures and molecular volumes of the pure components. One prediction of the theory, that the order parameters of the mixtures in the two-phase region are approximately independent of temperature, has been verified for binary mixtures of mesogens. In our case the two phase co-existence region, observed in mixtures of mesogenic and non-mesogenic materials, extends over a large temperature region but the behaviour of order parameter (S) falling more rapidly, is in contrast to that observed in mixtures of mesogens[2,3], where S remains constant.

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Effect of naphthalene on the frequency of moulting in speckled prawn, *Metapenaeus monoceros* (Fabricius)

(naphthalene/moulting/speckled prawn/*Metapenaeus monoceros*)

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ABSTRACT The effect of sublethal concentrations of naphthalene on the moulting frequency of the speckled prawn, *Metapenaeus monoceros* (Fabricius) has been studied. Inhibition of moulting was seen with all the concentrations tried and was concentration related.

Marine pollution caused by dissolved or dispersed hydrocarbons along the West Coast of India is of the order of 86.4 µg/l which is the highest in all world oceans[1]. In this situation, it is quite imperative to focus attention on the resident marine organisms, especially those harvested for human consumption. The speckled prawn, *Metapenaeus monoceros* (Fab.) is one of the commercially important species of Indian coast. Studies on toxicity of petroleum hydrocarbons suggest that naphthalene is one of the most toxic among them. The same was found to be evident during acute toxicity bioassays on *M. monoceros*[2]. Lockwood[3] has described factors which cause high natural mortality in moulting crustaceans. Hence, the impact of its sublethal concentrations during long term has been studied on moulting, since during moulting decapod crustaceans become more sensitive to natural environmental stresses.

Prawns were acclimated in glass aquaria as per the standard procedure[4]. Healthy prawns were randomly selected and exposed to naphthalene for 8 weeks. The concentrations employed were 0.75 mg/l, 1.5 mg/l and 3.0 mg/l which are below the 96-h LC_{50} level. Static bioassay procedure with 24-h water and toxicant replacement was employed. Acetone was used as the solvent for preparing stock solution of naphthalene. For control, acetone alone was added to the water. Prawns were daily supplied with tubificid worms *ad libidum* and were examined after every 24 hours up to a period of 8 weeks. Data on moulting frequency were obtained from the number of discarded exoskeletons found daily in each tank. Venkataramiah *et al.*[5] stated that the post-moult individuals often become victims of cannibalism, especially when reared in small spaces of aquaria. Therefore, care was taken to reduce cannibalism by placing coarse sand in each aquarium which provided shelter for the more sensitive, post-moult individuals.

A high degree of inhibition of moulting was seen in all the naphthalene stressed prawns (Table 1). Along with this, increasing naphthalene concentrations also cause greater mortality. Hence, values denoting reduction in percentage of moulting are less obvious. However, when the total incidence of moulting is considered, this reduction in moulting frequency is more obvious. All the same, there was still a

TABLE 1

Change in the moulting incidence during exposure of prawns to naphthalene.

Exposure period (Weeks)	Naphthalene concentrations (mg/l) 0.0 M	0.0 S	0.75 M	0.75 S	1.5 M	1.5 S	3.0 M	3.0 S
1	6	25	–	24	2	23	2	23
2	5	25	2	23	5	21	3	20
3	14	24	7	23	4	19	6	19
4	10	24	9	21	6	19	–	18
5	7	23	13	21	7	19	7	16
6	13	23	7	21	11	18	12	14
7	11	23	14	20	10	18	3	14
8	16	23	12	20	12	18	10	14
Per cent reduction in moulting frequency over control level			21.41%		30.48%		47.56%	
Total moultings over the weeks	82		64		57		43	

M = Number of moults
S = number of surviving prawns

reduction in rate of moulting. Thus the total moulting percentage during the entire 8 weeks of experimental period was 43% for surviving control prawns, 36% for prawns exposed either 0.75 mg/l or 1.5 mg/l of naphthalene and 31% in prawns exposed to 3.0 mg/l naphthalene. It is apparent from these data that exposure to long term sublethal concentration of 3.0 mg/l of naphthalene reduces the moulting frequency of prawns by 28% below the control levels.

It was also observed that the inhibition of moulting reflected on growth which was evident in all experimental groups but is concentration dependant. Similar inhibitary effect on growth and/or moulting rate in larvae or adults has been reported for many other crustacean species exposed to different types of pollutants[6-17].

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Micronucleus test (MNT) in four species of fishes treated with the bacterium, *Pseudomonas aeruginosa*

(fishes/*Pseudomonas aeruginosa*/micronucleus test)

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ABSTRACT The micronucleus test (MNT) has been conducted in peripheral erythrocytes and cells of kidney and gills of three Indian major carps, *Labeo rohita* (rohu), *Catla catla* (catla) and *Cirrhina mrigala* (mrigal) and the exotic fresh water mouth brooder, *Oreochromis mossambicus* (tilapia) for assessing the clastogenic potentiality of the bacterium, *Pseudomonas aeruginosa* after intraperitoneally injecting the fishes with the log culture against nutrient broth injected control. The qualitative and the quantitative assessment of the micronucleated erythrocytes (MNE) in peripheral blood smear in all the species revealed positive but differential responses as compared to control. Micronucleated cells (MNC) are found in gills and kidneys. The present study is new to the field on the effect of 'living mutagens' in fishes.

That the MNT is a relatively quick dependable method for screening genotoxic potentialities of chemical, physical and living mutagens[1] in bone marrow cells of mice needs no emphasis. In the recent past using fish as an aquatic model, Hooftman and Raat[2] studied the nuclear anomalies in peripheral erythrocytes of eastern mudminnow, *Umbra pygmea* after treating with EMS and Manna *et al.*[3-5] carried out MNT in peripheral erythrocytes, and in cells of kidney and gill of tilapia after treating with X-rays and some chemicals. The mutagenic, carcinogenic and teratogenic potentiality of the bacterium, *P. aeruginosa* in mice systems[6] and the epithelial carcinoma in fish, *Anabas*[7] has been reported earlier. In this paper the clastogenic potentiality of *P. aeruginosa* in erythrocytes, gill and kidney cells of treated specimens of four species of fishes detected by the MNT is being reported. It is a part of the programme undertaken by the present authors to study the effects of 'living mutagens' on fishes.

Three specimens, individually weighing between 30 g and 50 g, each of three Indian major carps, *Labeo rohita* (rohu), *Catla catla* (catla) and *Cirrhina mrigala* (mrigal) and the exotic mouth brooder, *Oreochromis mossambicus* (tilapia) weighing between 25 g and 30 g were intraperitoneally injected with the log-culture of the bacterium, *Pseudomonas aeruginosa* at the rate of 1 ml/100 g body weight per individual in the treated series while other sets of normal specimens of four species injected at the same rate with the freshly prepared nutrient broth served as controls. After 24 h of the injection the peripheral blood of each control and treated specimen was smeared separately on grease free clean slides. Next day air dried blood-smear slides were fixed in methanol for 3 min. Some slides were stained with Wright's blood staining solution and others with May-Grunwald's eosine methylene blue Giemsa staining schedule. After staining with Wright's stain for 5 minutes, the stain on slide was diluted with distilled water and was kept for 15 min. The slide was then rinsed in distilled water and was allowed to dry in air. The stained slides

were scanned under oil immersion randomly to determine the number of micronucleated erythrocytes among 2000 cells of each individual of 4 species of control and treated series.

The normal mature erythrocytes in all the species were of elliptical shape with a nucleus at the middle. There was generally one very small micronucleus in MNE lying close to the main nucleus in all the four species treated with log culture of *Pseudomonas* (Figs. 1–9). Rarely more than one micronucleus in MNE was encountered (Fig. 10). In the control micronuclei were practically absent as only 2 out of the 6000 erythrocytes examined in catla had a micronucleus (Table 1). The average frequency of

TABLE 1

Frequency of micronucleated erythrocytes (MNE) in peripheral blood of four species of fishes injected with the log culture of *P. aeruginosa* against nutrient broth injected control.
(Dose used 1 ml per 100 g body weight)

Species	No. of indiv.	Series	No. of erythrocyte	No of MNE	% of MNE
Labeo rohita	3	Control	6000	0	0
	3	Trented	6000	35	0.58
Catla catla	3	Control	6000	2	0.03
	3	Treated	6000	36	0.60
Cirrhina mrigala	3	Control	6000	0	0
	3	Treated	6000	11	0.18
Oreochromis mossambicus	3	Control	6000	0	0
	3	Treated	6000	19	0.32

MNE in 3 specimens was 0.58% in rohu, 0.60% in catla, and 0.18% in mrigal and 0.32% in tilapia (Table 1), indicating the differential responses in different species of fishes to the mutagenic effect of the *Pseudomonas* culture. It was lowest in mrigal and highest in catla (Table 1).

The MNT was carried out in the cells of kidney and gills of same individuals of four species of fishes treated with the log culture of *P. aeruginosa* against nutrient broth injected controls. The preliminary study showed that these two tissues also contained micronucleated cells in treated series (Figs. 11–14), the frequency study of which is in progress. From these preliminary studies it appears that cells of gills have relatively more micronucleated cells It needs to be ascertained whether this is an artifact due to the presence of bacterial cells in gill surface in normal state or in the treated specimens the degeneration of fragmented mitotic cells of kidney and gills leads to the appearance of variable number of pycnotic blocks due to the degenerating chromosomes (Figs. 12-14). Further studies are in progress.

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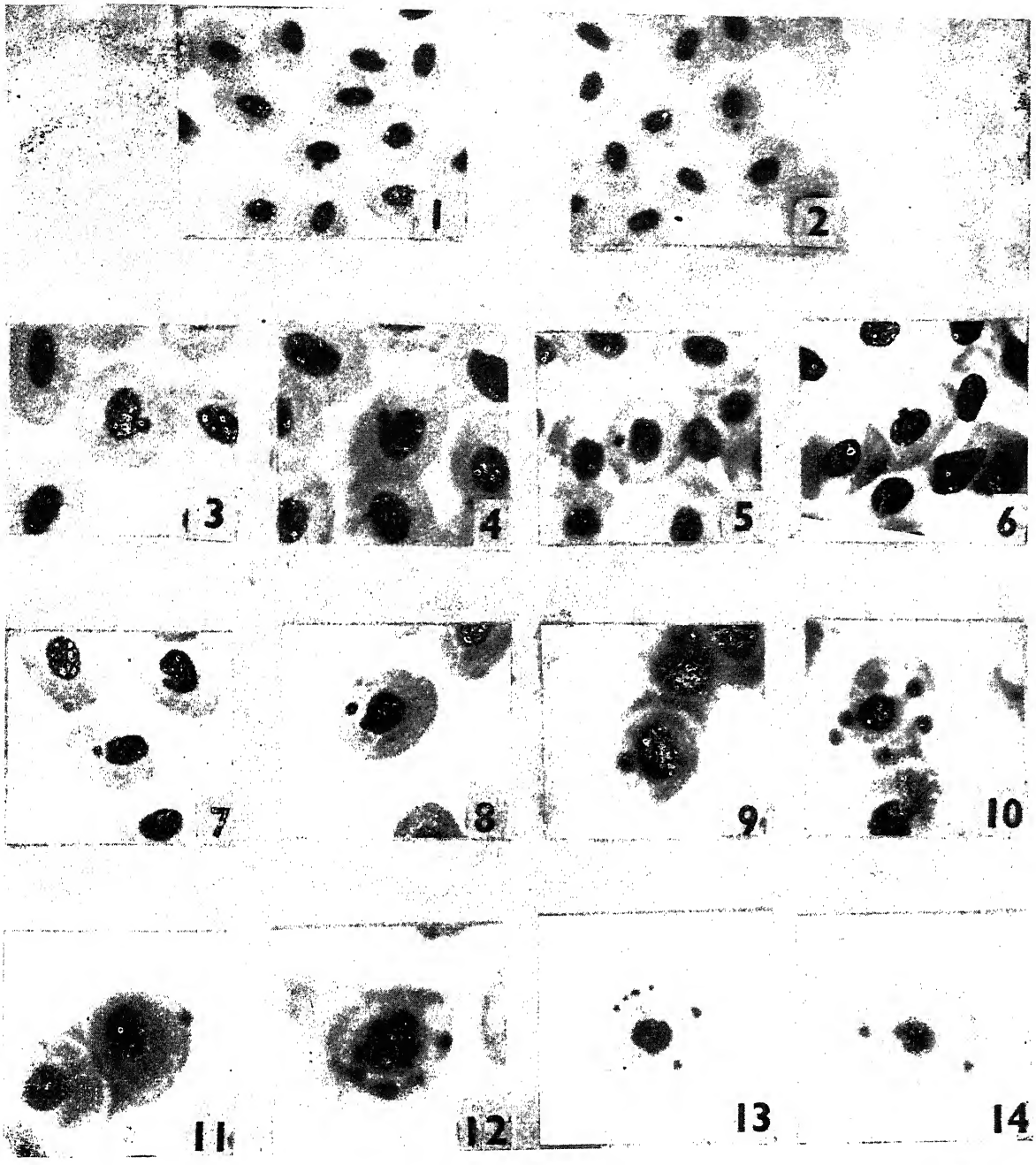

Figs. 1–14 Showing generally one micronucleus per micronucleated cell in erythrocytes (Figs. 1–10), gills (Figs. 11–13) and kidneys (Fig. 14) of four species of fishes injected with log culture of the bacterium *P. aeruginosa*. Figs. 1 & 2. general view of normal and micronucleated erythrocytes in *L. rohita* and magnified view of mainly micronucleated erythrocyte of *L. rohita* (Figs 3 & 4), *C. catla* (Figs. 5 & 6), *C. mrigala* (Figs. 7 & 8) and *O. mossambicus* (Figs. 9 & 10) and magnified view of micronucleated cells in gills of *C. Catla* (Figs. 11 & 12) and *O. mossambicus* (Fig. 13) and in kidney of *O. mossambicus* (Fig. 14). Exceptionally some cells showing more than one micronucleus (Figs. 10, 12-14) could be due to other reasons.

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The Indian Physical Society is planning to organise the 4th Colloquium for Young Physicists† on August 19-20, 1986 at Saha Institute of Nuclear Physics, Calcutta-700 009. It is planned to have about twenty lectures of 30 minutes duration each. There will be three awards for the best presentation as adjudged by a Board of Judges.

The Society invites young physicists and their research groups to submit papers to the General Secretary by July 21, 1986. The work should have been mainly carried out in India. The decision of the salection committee for papers will be notified by the 1st week of August, 1986. The authors of selected papers will be requested to present a 30 minutes talk reviewing their field of research along with their contribution.

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15 May 1986

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(a) Each communication may not contain more than 1500 words. It should be preceeded by an 'Abstract' of not more than 50 words.

(b) Manuscripts should be typewritten in English, double spaced and should be submitted in duplicate. All mathematical expressions should be typed or written clearly in black ink.

(c) The communication should contain a minimum number of tables, figures and photographs. Figures must be drawn in such a way that they can be reduced to one column width (7.5cm). Figures must be original drawings or exceptionally sharp glossy prints of about manuscript size. The space occupied by the figures/tables/photographs will be at the expense of text, because no LETTER which occupies a space more than 1500 words space may be published.

(d) All references should be indicated in the text by superscript arabic numerals, e.g. "Mirri[2] while working on.. ". The list of references should be arranged in order of their occurrence in the text. Reference should be given in the following style :

2. MIRRI, M. A. (1982) *J. Chem. Phys.* 58 : 282. (For articles in Journals).
   Author year Journal vol. beginning page

   White, M. J. D. (1973) *Animal Cytology and Evolution*, 3rd Ed., Cambridge University Press, London, p. 320 (For Books).

   Osgood, C. F. (1977) in *Number Theory and Algebra*, ed. Zassenhaus, H., Academic Press, New York, p. 321. (For edited Books)

   Abbreviations of the names of periodicals should conform to those given in the World List of Scientific Periodicals.

(e) Key words : A maximum of 5 key words should be supplied. Each of these words will be separated by a slash (/) and printed just below the title of the research paper, e. g. (steroid receptors/protein-DNA interactions/gene regulation).

(f) Acknowledgements, if any, should appear at the end of the letter, but just before the references.

(g) Proofs will not ordinarily be sent to authors. If more than 100 reprints are needed, indicate the extra number while sending the manuscript.

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